## — SECTION D —

## ZOOLOGICAL SCIENCES

## Contents

## THE EFFECT OF TEMPERATURE ON THE GROWTH AND EFFICIENCY OF YOLK CONVERSION IN THE SALMON EMBRYO[1]

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### Abstract

Salmon larvae from one female were placed in a series of 12 temperature chambers ranging from 0.2° to 16° C., at approximately the time of hatching. The changes in weight of the embryo and yolk sac were followed for some time, and finally brought to a common value representing the embryo gain (or yolk loss) in 10 days. From the results were calculated the temperature coefficient, $Q_{10}$, for embryo growth, which showed a drop in the colder chambers from about 8 to a little over 2 at 8°, the latter being maintained at warmer temperatures. When the $Q_{10}$ for activity (yolk loss minus embryo gain) was worked out it proved to follow the same plan as the growth values just mentioned. The effect of temperature on efficiency was also worked out. Efficiency is defined as

$$\frac{\text{dry embryo gain} \times 100}{\text{dry yolk loss}}.$$

Efficiency in the cold chambers was found to be low and constant at some 42%. At 5° it began to rise, to reach a maximum of nearly 60% in the warmest chamber.

In the natural life of a salmon the eggs are fertilized some time early in November, the water being a few degrees above the freezing point. From then on the water drops gradually to just above freezing, in the bottom of the rivers, and development is slowed down. Later on, with the onset of spring, the water begins to warm up, with a consequent speeding up in the developmental processes. What emerges from all this is a normal embryo, of characteristic size, depending on the size of the egg at the start. For such an embryo it would be expected that there would be a proper set of proportions; for instance there ought to be, from one year to the next, a similar ratio between length and number of somites, or between length and weight, or between yolk used up and embryo produced. Energy might be expected to be used up in some regular way for the three great demands of development, namely, growth, differentiation, and the maintenance of material already formed. In a word the processes of development proceed at a rate calculated to produce an embryo with the maximum possible chance of survival. It might be that alterations of temperature would not operate on all phases of development in the same way, and that by the application of a series of temperatures to embryos as they developed, one could dissociate the funda-

mental processes of ontogeny.   Thus at unusual temperatures parts might not differentiate in the usual order, or growth might not stand in the typical relation to differentiation, i.e. an embryo might be too big for its degree of development.   There is, of course, a limit to the extent to which the processes of development can be uncoupled without killing the embryo.   For example, as Tyler (17) points out, if a chick embryo were kept at the one somite stage while enlarging to the size attained at hatching, the lack of a proper circulation would render the food material unavailable except to those cells adjacent to the yolk.   Oxygen would also become unavailable except to the surface cells.

The object of the investigation, of which this paper forms a beginning, is to find out whether the normal order of embryological events can be altered by temperature changes, and, if so, to what extent the alteration can go while leaving the developing embryos alive.   The requirements are first, facilities for rearing eggs at different temperatures, second, facilities for measuring growth, and third, facilities for measuring differentiation.   These may be considered in turn.

A series of temperatures was provided by use of a heavily insulated differential thermostat constructed for the purpose.   The instrument consisted essentially of a trough made of copper $\frac{1}{8}$ in. thick, which was divided by partitions into compartments.   The trough was led at its ends into tanks filled with fluid, one of which was provided with a heating arrangement and the other with the coils of an electric refrigerator.   Each end was controlled within 0.5° C. and the experimental chambers took up a graded series of temperatures, which are shown in Table I.   In these air chambers the larvae

TABLE I

WEIGHTS ARE OF ONE SPECIMEN IN MILLIGRAMS.   THE FIRST HORIZONTAL ROW (IN WHICH TEMPERATURE IS REPRESENTED BY *) GIVES THE VALUES AT THE START OF THE EXPERIMENT.   ALL OTHER WEIGHT VALUES ARE GIVEN AS OF 10 DAYS LATER.   ALL LARVAE USED WERE FROM ONE FEMALE

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| Temperature, ° C. | Embryo dry weight | Embryo dry gain | Embryo wet weight | Embryo wet gain | Yolk dry weight | Yolk dry loss | Yolk wet weight | Yolk wet loss |
| * | 3.78 | 0.00 | 30.2 | 0.00 | 39.4 | 0.00 | 87.5 | 0.0 |
| 0.2 | 4.33 | 0.55 | 33.5 | 3.39 | 38.1 | 1.3 | 86.1 | 1.4 |
| 2.8 | 4.92 | 1.14 | 36.2 | 6.14 | 37.0 | 2.4 | 83.0 | 4.5 |
| 4.0 | 4.75 | 0.97 | 38.0 | 7.94 | 36.7 | 2.7 | 81.7 | 5.8 |
| 6.3 | 5.45 | 1.67 | 42.9 | 12.8 | 35.3 | 4.1 | 80.2 | 7.3 |
| 8.4 | 6.1 | 2.3 | 49.8 | 19.7 | 34.8 | 4.6 | 78.3 | 9.2 |
| 9.3 | 7.1 | 3.3 | 41.8 | 16.7 | 35.0 | 4.4 | 68.8 | 18.7 |
| 10.6 | 6.8 | 3.0 | 60.6 | 30.5 | 33.5 | 5.9 | 74.6 | 12.9 |
| 12.2 | 6.9 | 3.1 | 62.6 | 32.5 | 31.7 | 7.7 | 70.8 | 16.7 |
| 13.3 | 7.9 | 4.1 | 63.6 | 33.5 | 32.1 | 7.3 | 73.1 | 14.4 |
| 14.3 | 8.1 | 4.3 | 68.6 | 38.5 | 28.9 | 10.5 | 63.5 | 24.0 |
| 15.2 | 8.9 | 5.1 | 75.6 | 45.4 | 30.8 | 8.6 | 65.4 | 22.1 |
| 16.0 | 7.2 | 3.4 | 71.9 | 41.7 | 30.6 | 8.8 | 66.5 | 21.0 |

were placed in flat-bottomed dishes containing $\frac{1}{2}$ in. of water. The specimens were placed in such a concentration that each had about $\frac{1}{3}$ cu. in. of water. Oxygen diffused through the water at a rate sufficient to meet the needs of the salmon, a matter established by a preliminary experiment in which eggs were hatched in the warmest chambers. Some caution is necessary in dealing with eggs for, as no stirring movement takes place, the delivery of oxygen is solely by diffusion. Diffusion is little affected by temperature, while the needs of the eggs are markedly increased by higher temperatures. It therefore might happen that too little oxygen would be delivered to the eggs in the warmer chambers, even though the conditions appeared alike in all chambers. It is important for this reason to keep the layer of water, in which the eggs are developing, as thin as possible. Rugh (16) has an interesting illustration of the effect of crowding on the size attained by frog tadpoles.

Were the larvae in the warmest chambers receiving sufficient oxygen to permit maximum growth? The only absolute proof would be to repeat the experiment under oxygen instead of air. The following considerations, however, make it probable that the oxygen supply was adequate.

1. The only stage at which oxygen lack at high temperatures has been reported as a critical factor is immediately before hatching, when the embryo within is of maximum size and the egg, being spherical, presents a minimum surface (5).

2. The present experiments began after hatching, which meant that the oxygen no longer had to diffuse through the capsule and perivitelline fluid to reach the embryo, and also that the surface was greatly increased by deviation from the spherical form.

3. The continuous swimming movements of the larvae, especially in the warmer chambers, caused the water to be stirred so that there would be no low-oxygen pockets around the specimens and no oxygen gradient from surface to bottom of the water.

Growth is taken here to mean an increase in size, either wet or dry. In the interval under consideration the percentage of water in the embryo did not change significantly, hence the wet weight is a simple multiple of the dry weight. It is therefore unnecessary to consider both, and the dry weight values have been selected for treatment. All specimens were weighed fresh, none formalin preserved. It is quite easy to separate the embryo from the yolk sac with forceps, at the ages used. The specimen was then placed for weighing on a small tared and numbered triangle of light weight waterproof paper (the kind that butter is wrapped in) with a hole punched through one end. After weighing, the paper with embryo was hung on a hook made from a bent pin, dried overnight at 95° C. and weighed again. The torsion balances used were made in the Department from a considerably modified design of Fabergé (3). To weigh a specimen required only 10 to 15 sec., so that there was no appreciable loss of water in the first weighing, or uptake of moisture from the air after removal from the oven in the second. The system of hanging the weighing papers on hooks also prevented loss of material by

leakage through to the substratum. Usually five embryos were weighed individually and the result averaged. Sometimes 10 or more were used. The recording of both wet and dry weights provided a check against an individual error in either of the readings, for the wet and dry curves could be compared at any desired point. All the results have been given as though they were taken 10 days after the beginning of the experiment. Actually the reading was taken at exactly 10 days in only one case—others were made at various times, and brought, by arithmetic, to what they would have been at 10 days. The warmer chambers, from 9.3° on, were observed within a day or two of 10 days. With the colder chambers, however, it was found that the increase of embryo (or loss of yolk) in this interval was so small that the errors of observation became considerable. Therefore it was decided in these cases to use later readings, taken respectively (from the cold end) at 83, 64, 114, and 34 days after the start of the experiment.

For example, after 83 days the dry weight of an embryo at 0.2° was found to be 8.40 mgm. If the dry weight at the start be taken as 3.78 mgm. (average of 20 initial weighings) the difference of 4.62 mgm. will represent the growth in 83 days. The growth in 10 days would therefore have been

$$\frac{4.62 \times 10}{83} = 0.55 \text{ mgm.}$$

and the theoretical weight after 10 days (as set down in Column 2 of the table) would be $3.78 + 0.55 = 4.33$ mgm.

By the equation it is assumed that growth in weight is a linear function of time whereas it is actually a parabolic function (6). But as inspection of Fig. 2 of (6) shows, the error involved will be negligible for the first two-thirds of the posthatching period, when the tests were carried out. In the present work the two or three weighings at each temperature give no clear evidence as to the shape of the growth curve.

The measurement of differentiation has been a difficult matter. Minot (8) proposed many years ago to take the proportion of actively dividing cells as a criterion, and his ideas have been followed recently by Richards (13, 14, 15). The method, however, is laborious, since sections must be made at all stages, and is really applicable only to quite young embryos. For use in this work a special series of salmon stages have been worked out (10). When the experiment was started the salmon were in Stage 13, which in this instance coincided approximately with hatching. (Hatching itself is not regarded as a stage, for there is some evidence (4) that it occurs quite variably). Stage 13 is characterized by the appearance of fin rays in the tail with an accompanying loop of blood vessels. The plan of the experiment was to measure the time of development from here to Stage 14 at each of the temperatures and thus to obtain a measurement of the rate of differentiation. In this we were unsuccessful for two reasons. In the warmest of the chambers the larvae did not live long enough to attain Stage 14, for evidently the temperatures were too high to sustain life permanently. Secondly, the very problem under investigation, namely, the uncoupling of embryonic processes intervened as a

disturbing factor.    Stage 14 was drawn up on the basis of observations on
embryos developing in water of an intermediate temperature, perhaps 6° on
the average.    In this thermal environment several morphological events
happened together, which are described and illustrated (10), and these events,
in the aggregate, constitute Stage 14.    For instance the fat cells in the
mesentery are first noticed at the same time that the gut has become turned
through a right angle.    But in the coldest of the experimental chambers the
gut never turned through a right angle although the fat multiplied in the
mesentery to the point equivalent to Stage 15 as originally described.    On
the other hand in the warmer chambers the gut turned through a right angle
and development proceeded until the conspicuous pigment patches illustrated
in Fig. 15a (10) appeared, but during all this time no fat ever appeared in the
mesentery.    Evidently therefore temperature is capable of producing an
alteration in the order of morphological events, and in this particular case
we were not able to fix on a date that could be taken with confidence as
Stage 14.    No attempt will be made therefore, in this paper, to compare the
rate of differentiation with the rate of growth, but attention will be restricted
to the latter.

Figs. 1 and 2 show respectively the change in quantity of dry embryo and
dry yolk in a 10 day period at each of the experimental temperatures, the
data being taken from Table I, Columns 3 and 7.    Semilogarithmic paper
has been used, which gives the same graphical effect as plotting the logarithms
of the weight changes against temperature.    Where straight lines appear it
will represent $Q_{10}$ (discussed below) to be unchanging over an interval.    The
low part of the temperature range on each graph is represented by a curved
line, the higher part by a straight line.    A little inspection will show that the
graph might have been fitted, without great change in the statistical prob-
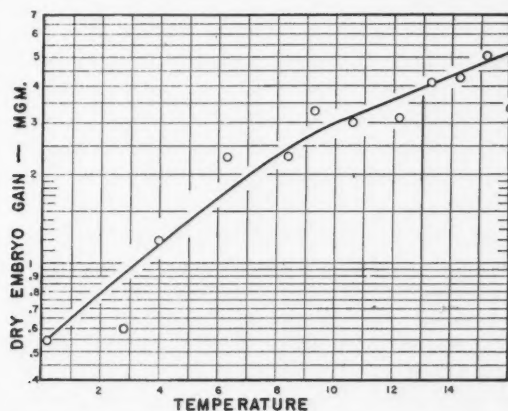


FIG. 1. *Gain in dry weight of a salmon embryo at various temperatures in the 10 day
interval following Stage 13 (approximately the 10 days after hatching). Weight increases
are plotted on a logarithmic scale, temperatures on an arithmetic scale.*

abilities, by two straight lines intersecting in approximately the middle of the range. The result of such graphical presentation would have been to represent the temperature quotient, when it came to be calculated, as constant
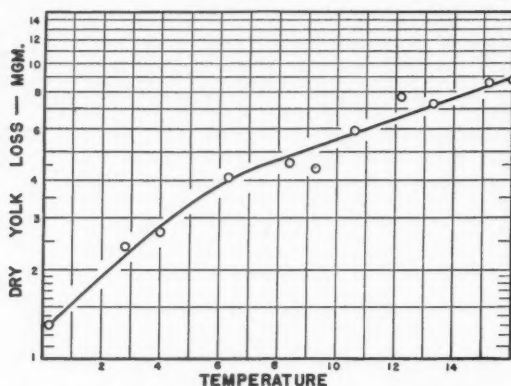


FIG. 2. *Loss in dry weight of yolk sac in the same 10 day interval covered by Fig. 1. Weight losses on logarithmic scale, temperatures on an arithmetic scale.*

over each of the ranges represented by a straight line with a sudden drop at the intersection. Such a treatment has actually been given to results on trout by Embody (2) and whitefish by Price (12). Nevertheless it appears biologically incredible that such a complex system as a developing embryo should change its temperature characteristics with a jump, and the straight line method of graphs is to be justified only on the grounds that matters are thereby eased for the statistician—grounds that we consider insufficient.

About the only comments necessary of Figs. 1 and 2 are that (as might have been predicted) changes take place more rapidly at higher temperatures, and that the effect of temperature appears at a glance to be the same on yolk loss as on embryo gain. That it is not quite the same, however, appears from Fig. 3, in which the efficiency of the process of conversion of yolk to embryo is shown. Efficiency, expressed as a percentage, is defined as

$$\frac{\text{embryo gain} \times 100}{\text{yolk loss}}.$$

Fig. 3 shows that in the cooler chambers efficiency is at a minimum of less than 42%, which means that more than half of the yolk is used up in the process of converting the rest into embryonic tissues. The figure remains constant at its low value up to approximately 5° after which it begins to rise, and continues to rise until the warmest temperatures, at which a maximum of nearly 60% is reached. The series of values falls in the same range as those obtained by previous workers, as reviewed by Needham (9, pp. 934-939). See also (5). In the construction of Fig. 3, and also in Figs. 4 and 5 to follow, the graph has been drawn from points read off the smoothed curves of Figs. 1 and 2.
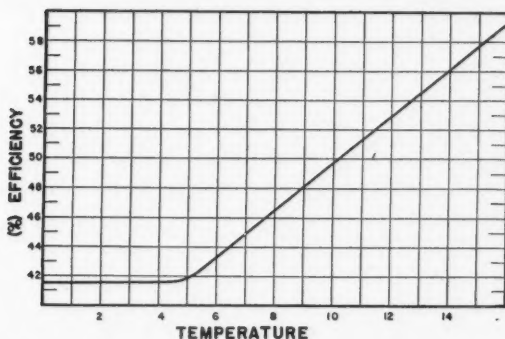
FIG. 3. *Effect of temperature on the efficiency of conversion from yolk to embryonic tissue, calculated from the dry weight values read off the curves of Fig. 1 and Fig. 2. Efficiency is defined as* 100 × *embryo gain divided by yolk loss.*

Figs. 4 and 5 deal with temperature coefficients. We have used the familiar $Q_{10}$ nomenclature in which

$$\log Q_{10} = \frac{10 \ (\log K_1 - \log K_2)}{t_1 - t_2}$$

where $K_1$ and $K_2$ represent embryo gain (or yolk loss, etc.) at temperatures $t_1$ and $t_2$. Fig. 4 shows the course of the coefficient for embryonic growth and it shows a decline from the coldest chamber until about 10° or more, following which it appears to flatten out. There is little doubt that the quotient is
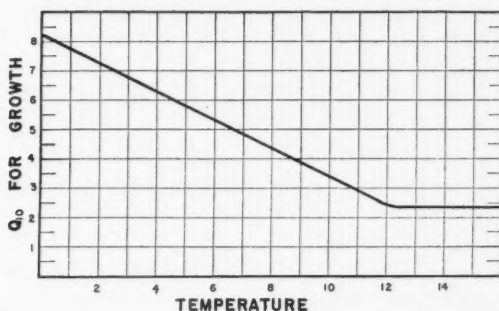


FIG. 4. *The temperature coefficient, $Q_{10}$ for growth as plotted from the smoothed embryo growth values in Fig. 1.*

lower at the warm end than at the cold, but the values are not precise enough to show whether the mechanism of reduction is exactly as shown or not. The beginning values of nearly 8 are somewhat higher than is usual for biological systems; the later ones are more in the usual line. It is customary for $Q_{10}$ to be higher in the cool end of the biological range, so that is nothing especially remarkable about this part of the matter.
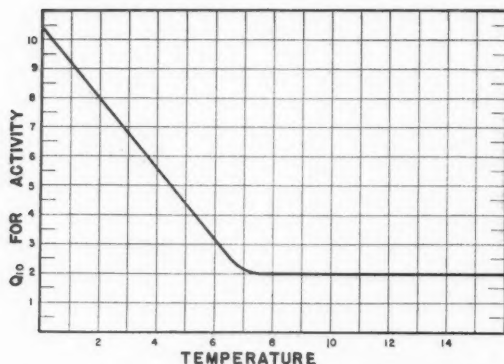
FIG. 5. $Q_{10}$ for activity as plotted from smoothed curves of Figs. 1 and 2.  It is assumed that yolk loss minus embryo gain represents material used up for activity.

It would be instructive to know whether a difference could be demonstrated between $Q_{10}$ for growth, as discussed in the preceding paragraph, and $Q_{10}$ for maintenance.   One might expect a difference, since there is, at higher temperature, an obvious increase in activity, such as swimming movements, which does not necessarily correspond with an increase in growth.  Values may be obtained for the yolk used up for maintenance by subtracting the embryo gain from the total yolk loss.   From the resulting series a $Q_{10}$ curve for maintenance can be plotted (Fig. 5).   On examination Fig. 5 turns out to be indistinguishable, within the limits of error, from Fig. 4, which leads to the conclusion that growth and maintenance are affected in the same manner by temperature changes.

Any of the foregoing $Q_{10}$ values may be converted to the $\mu$ values of the Arrhenius equation with negligible error (5%) if multiplied by 5500.   The reason that a 5% error is negligible is because all temperature coefficients are inherently of low accuracy by reason of the nature of the calculations used.  As the equation shows, the numerator is formed by subtracting from a large weight, a slightly smaller weight; the denominator is similarly formed by subtracting from one temperature reading a slightly smaller one.   The errors of all four readings are increased and heaped on the quotient.   This matter has been discussed with reference to other calculations by Hayes and Armstrong (6).   The reason that a simple conversion factor can be used to get $Q_{10}$ into $\mu$ is that for a short temperature range the reciprocals of the absolute temperatures as used in the Arrhenius plot form approximately a linear series themselves.   Thus the plot of log rate vs. temperature (for $Q_{10}$) becomes the same, within experimental error, as the plot of log rate vs. reciprocal of temperature (for $\mu$).   Finally it should be noted that the theoretical validity of the Arrhenius equation when applied to biological material has been questioned with some vigour by various authors (1, 7, 11).

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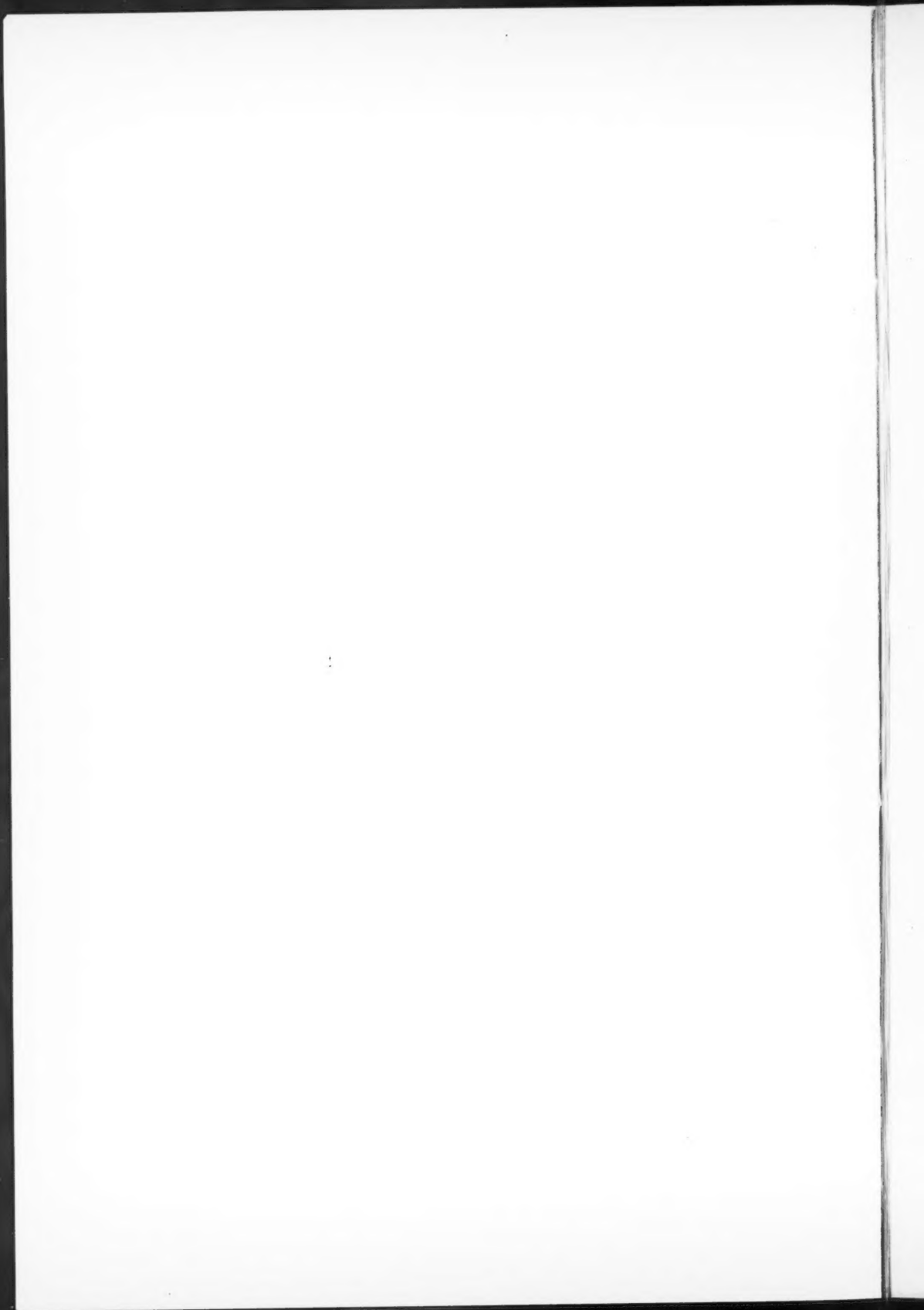
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